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Nutritional Content of *Artemia* sp. Fed with *Chaetoceros calcitrans* and *Skeletonema costatum*

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***Artemia* sp. is a natural food with high protein content, especially amino acid. Nowadays, Indonesia still relies on import for its supply. Hence, the utilization of local *Artemia* sp. as an alternative to the imported product is recommended as it contains more protein and less expensive. The advantages of local *Artemia* sp. is its better crystal quality as it is still fresh. It also provides better income for salt farmers because waste water from salt farm can be used to culture *Artemia* sp.. This research is aimed at determining the quality of locally-produced *Artemia* sp., by evaluation of its essential amino acid and fatty acid profiles after treatments. Our results indicated that *Artemia* sp. cysts with good quality were produced after 8 hours and *Artemia* sp. reaches a hatching rate of 1,320,000 cysts (95%) after 27 hours. We also found an indication that the best feed concentration was a mix of 60% *Chaetoceros calcitrans* with 40% *Skeletonema costatum*. Fatty acid profile analyses showed that the highest SAFA (12.86%) and PUFA (29.91%) were gained after feeding with *Chaetoceros calcitrans*, whereas the highest HUFA (4.93%) was gained after feeding with *Skeletonemacostatum*. Essential amino acid profile analyses revealed the highest content of amino acid (18912.62 ppm) was after feeding with a combination of *Chaetoceros calcitrans* and *Skeletonema costatum*. Finally, the proper water quality during research was at 25-30 °C of temperature, 30-31 ppt of salinity, pH 7.8-8.9, and DO was at 3.0-4.4 mg/L.**

Key words: locally-produced *Artemia* sp., *Chaetoceroscalcitrans, Skeletonema costatum,* essential amino acid profile, fatty acid profile

INTRODUCTION

One of the key factors for successful shrimp and fish farming is the availability of feed at the proper amount and size for the right stadium of fish and shrimp. Quality feed at the right amount will ensure the survival of shrimp larvae (Herawati 2013). One of the main zooplankton used as natural feed for fish and shrimp farming is *Artemia* sp., as it contains high amount of protein and amino acids (Vilchis 2010). But, the demand for *Artemia* sp. in Indonesia is currently supplied by imported product, despite the fact that it is possible to obtain it locally. Imported *Artemia* sp. contains 70% protein and 6.3% fat, while locally-produced *Artemia* sp. has 79.91% protein and 8.6% fat (Sudaryono 2006; Mintarso 2007; Suhartanto *et al*. 2008). Moreover, locally-produced *Artemia* sp. possesses better quality of cysts as it is still fresh, and it actually provides extra earning for salt farmers if they are willing to culture it (Mintarso 2007; Herawati 2013).

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Significant attention has not really been given to the culture of locally-produced *Artemia* sp. in salt farms (Mintarso 2007). This might be related to the common assumption that locally- produced *Artemia* sp. is of low quality, due to its low fatty acid and amino acid content. Therefore a thorough research to improve the nutritional content of *Artemia* sp. must be conducted in order to get higher fatty acid and amino acid in *Artemia* sp.

Improving the content of essential amino acid and fatty acid in *Artemia* sp. requires quality of feed that is rich in protein that will in turn produces high quality *Artemia* sp. (higher hatching ability). Feed plays a major role in producing high quality *Artemia* sp. (Herawati *et al*. 2012). As feed for fish and shrimp larvae, *Artemia* sp. itself needs proper intake to improve its nutritional content, especially protein and fat (Widiastuti *et al.* 2012). *Artemia* sp. lacks of EPA and DHA zooplanktons, which have essential characteristics (Riberio & Jones 2008). Therefore *Artemia* sp. needs external enrichment of high EPA and DHA content.

A research by Hoa *et al.* (2011), mentioned that *Chaetoceros gracilis* serves the purpose of improving

the composition of nutrition and biomass of *Artemia* sp. which was cultured in lake. They further emphasized that after being fed with *Chaetoceros gracilis*, the protein content in *Artemia* sp. was at 40-55%, and the fat content was at 4-6%, whereas the values for EPA and DHA were not identified. Nonetheless, no accurate measure of the amount of *Chaetoceros gracilis* given in their research. The only parameter used was change in the color of lake; dark brownish color means that *Chaetoceros gracilis* was still available, and clear water means that the *Chaetoceros gracilis* supply was no longer available. Hence, studies on the proper dose of *Chaetoceros gracilis* for locally-produced *Artemia* sp. are of great interest and important. *Skeletonema costatum* and *Chaetoceros calcitrans* contain adequate nutrition (Anderson 2005; Herawati *et al.* 2012). They can be used to improve the nutritional content of *Artemia* sp. Moreover, their dimension is so tiny (less than 60 µm) that makes them edible for *Artemia* sp. (Herawati 2013). Once properly fed, locally-produced *Artemia* sp. will in turn ready to be fed to fish and shrimp larvae.

In an effort to improve the nutritional content of *Artemia* sp., as observable from the essential amino acid and fatty acid profiles, this research devised an alternative feeds: *Chaetoceros calcitrans*, *Skeletonema costatum*, and a combination of both. Our results are useful for shrimp and fish hatcheries on the proper feeding of locally-produced *Artemia* sp., in order to reduce over dependence on its imported counterpart.

MATERIALS AND METHODS

This research was experimental in nature. The material used was locally-produced *Artemia* sp. in Lasem, Rembang regency. The diatom seeds or *Chaetoceros calcitrans* and *Skeletonema costatum* were obtained from the Natural Feed Laboratory of BBPBAP Jepara, which were then mass-cultured using the Guillard culture medium (Herawati *et al*. 2012).

Equipment preparation and sterilization followed Hoa *et al.* (2011), that tools should be washed and put into the oven. As for containers, they were washed, brushed, and then dried under the sun.

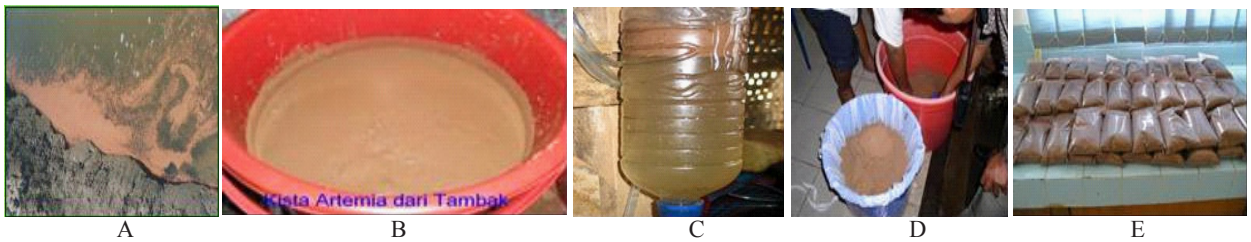
***Artemia* sp. Production Technique.** The technique for *Artemia* sp. production followed Mintarso (2007) and Herawati (2013), as depicted in Figure 1. The *Artemia* sp. that ready for cultivation was located on the edges of the farm (Figure 1A), which then filtered with a layered filter to separate cysts from mature *Artemia* sp. (Figure 1B). The next step was a separation of cysts from other dirts, and conditioning of cysts so they will not hatched at high salinity sea water (250-300 ppt) (Figure 1C). Afterwards, the cysts were packed into 500 g per unit and then sold for Rp. 400,000 per kg (Figures 1D and E).

***Artemia* sp. Feeding Method.** Feeding for *Artemia* sp. was conducted at instar 2. The method for feeding utilized the Complete Random Design that consisted of 3 treatments, each with 3 repetitions. These treatments were feeding with 100% *Chaetoceros calcitrans,* feeding with 100% *Skeletonema costatum,* and feeding with a combination of 60% *Chaetoceros calcitrans* and 40% *Skeletonema costatum*. The combined feeding was based on the 34.02% *Chaetoceros calcitrans* and 28.53% *Skeletonema costatum* protein contents (Herawati *et al*. 2012; Herawati 2013).

***Artemia* sp. Density.** Locally-produced *Artemia* sp. will hatch into instar 1 in about 8 hours. They were then transferred into research containers with a density of 200 cysts/liter (Mintarso 2007). The naupli were calculated using the Hidgdon method (2010) as follows: (i) the hatched nauplii were separated from their shells and then transferred into a beaker glass that contained 100 mL sea water; (ii) using a pipette, 1 mL of nauplii was taken from that glass and transferred into a beaker glass that also contains 100 mL of sea water; (iii) final step, 0.5 mL of nauplii was taken from the second glass and the number of nauplii (H) was calculated using the Treece formula:

$$G = (H) \times 1000$$

the result was nauplii density. The next procedure was feeding of hatched *Artemia* sp. after 12 h for the next 7 days which has become a young adult. The density of *Chaetoceros calcitrans* and *Skeletonema*



A B C D E

Figure 1. Technique for the production of locally-Produced *Artemia* sp. (Herawati 2013).

costatum was provided as feed based on the feeding pattern of *Artemia* sp. with *Dunaliella* sp., which was at 10^5 (Herawati *et al*. 2012).

***Artemia* sp. Quality.** The quality of *Artemia* sp. was readily observable from its hatching rate. It is very much depend on the hatching, from the moment it is a cyst into its nauplius (Riberio & Jones 2008).

Fatty Acid Profile. The fatty acid profile of *Artemia* sp. can be determined by analyzing its total fatty acid content. The equipment used for this purpose was a gas chromatograph with a W Cot fused Silica Counting CP-SIL-88 column of 50 m length, 0.22 mm diameter, and at a column temperature of 120-200 °C. The method employed was *in situ* transcertification (Park & Goins 1994). One hundred mg of *Artemia* sp. sample was homogenized using 4 mL of water. The resulting 100 µL homogenate was then transferred into a reaction tube. One hundred µL of methylene chloride was then added, along with 1 mL of NaOH 0.5 N in methanol. Once nitrogen was added and the tube was sealed, it was heated to 90 °C for 10 min. The reaction tube was then cooled and 1 mL of 14% BF3 in methanol was added. After nitrogen addition, heating ensued at the same temperature for the next 10 min. Afterwards, the reaction tube was cooled to ambient temperature, and 1 mL of water and 200-500 µL of hexane were added. The mixture was then vortexed for 1 min to extract the fatty acid's methyl ester. After centrifugation, the upper layer of sample was ready for GC analysis.

Essential Amino Acid Profile. The essential amino acid profile of *Artemia* sp. were determined by examining its essential amino acid content. Essential amino acid analysis was conducted by using an HPLC with a Eurospher 100-5 C18, 250 x 4.6 mm column that has P/N: 1115Y535 pre-column. The effluents were: (i) 0.01 M acetate buffer at pH 5.9; and (ii) 0.01 M MeOH acetate buffer at pH 5.9; THF> 80:15:5 Λ Fluorescence: Ext : 340 mm Em : 450 nm. About 2.5 g of sample was put into a sealed glass. Then, 15 mL of HCl 6N was added. The mixture was then vortexed for homogeneity and underwent hydrolysis using an autoclave at 110 °C for 12 hours, before being cooled down to room temperature and neutralized with NaOH 6N. After addition of 2.5 mL of 40% Pb Acetat and 1 mL of 15% oxalate acid, around 3 mL of the mixture was filtered with 0.45 µm millex. For the injection into HPLC, 25 µL of the filtered mixture plus 475 µL of OPAA solution was vortexed and incubated for 3 min. Finally, 30 µL of final mixture was put into the HPLC.

Data Analysis. The data gained from this research was the quality of *Artemia* sp. determined from its essential amino acid and fatty acid profiles after being fed with *Chaetoceros calcitrans* and *Skeletonema costatum.*

RESULTS

Water Quality. The water used for the research was within the proper quality and it is presented in Table 1. The temperature was between 25.0-30.5 °C, pH was around 7.8-8.9, the salinity was in the range of 30-31 ppt, and the DO was at 3.0-4.4 mg/L.

***Artemia* sp. Quality.** Observation of the locally-produced *Artemia* sp. showed that the culture started hatching period on the 9th hour with a hatching degree of 1.1% or 15,000 hatching cysts. Results for nutritional content based on proximate analyses for locally-produced and imported *Artemia* sp. are presented in Table 2. The results show that imported *Artemia* sp. has 43.33% protein and 6.96% fat, whereas locally-produced *Artemia* sp. contains 62.41% protein and 8.66% fat.

Fatty Acid Profile. Results for nutritional content based on fatty acid profile for locally-produced and imported *Artemia* sp. are presented in Table 3. Our analyses showed that the highest saturated fatty acid (palmitic acid) in *Artemia* sp. was gained after feeding with *Chaetoceros calcitrans* (12.86%). As for the highest unsaturated fatty acid (oleat), it was available when *Artemia* sp. was fed with *Chaetoceros calcitrans* (25.09%). The highest EPA in *Artemia* sp. was obtained when it was fed with *Skeletonema costatum* (4.93%). The fatty acid profile after feeding with both *Chaetoceros calcitrans* and *Skeletonema costatum* is given in Table 4.

DHA content was not available on the profile after feeding with both *Chaetoceros calcitrans* and *Skeletonema costatum*. Therefore, it can be inferred that feeding with both *Chaetoceros calcitrans* and

Table 1. Water quality

Parameter	Range	Standard
pH	7.8-8.9	8-9a; 8.0-8.4b
DO (mg/L)	3.0-4.4	4-5a; 3-4.5b
Temperature (°C)	25-30.5	25-30a; 25.6-30.5b
Salinity (ppt)	30-31.0	30-50a; 30-95b

a. Mahbub (2010), b. Vilchis (2011).

Table 2. Proximate analyses for locally-produced and imported *Artemia* sp.

Parameter	Locally-produced *Artemia* sp. (% ± sd)	Imported *Artemia* sp. (% ± sd)
Ash	7.79 ± 0.023	6.28 ± 0.016
Fat	8.66 ± 0.012	6.96 ± 0.028
Proteins	62.41 ± 0.029	43.33 ± 0.015
Crude fiber	6.03 ± 0.019	5.28 ± 0.033
Carbohydrate	15.08 ± 0.027	38.12 ± 0.036

Table 3. Fatty acid profile for locally-produced and imported *Artemia* sp.

Imported *Artemia* sp.			Locally-produced *Artemia* sp.		
Fatty acid methyl ester		%	Fatty acid methyl ester		%
Kaprilic fatty acid	C 8 : 0	-	Kaprilic fatty acid	C 8 : 0	-
Kapric fatty acid	C 10 : 0	-	Kapric fatty acid	C 10 : 0	-
Lauric fatty acid	C 12 : 0	0.07 ± 0.008	Lauric fatty acid	C 12 : 0	-
Miristic fatty acid	C 14 : 0	0.94 ± 0.015	Miristic fatty acid	C 14 : 0	3.74 ± 0.011
Palmitic fatty acid	C 16 : 0	7.92 ± 0.026	Palmitic fatty acid	C 16 : 0	9.14 ± 0.017
Palmitoleic fatty acid	C 16 : 1	12.34 ± 0.033	Palmitoleic fatty acid	C 16 : 1	9.84 ± 0.027
Stearat fatty acid	C 18 : 0	4.04 ± 0.018	Stearat fatty acid	C 18 : 0	3.80 ± 0.019
Oleic fatty acid	C 18 : 1	24.90 ± 0.009	Oleic fatty acid	C 18 : 1	18.98 ± 0.014
Linoleic fatty acid	C 18 : 2	5.64 ± 0.016	Linoleic fatty acid	C 18 : 2	2.87 ± 0.009
Linolenic fatty acid	C 18 : 3	6.10 ± 0.031	Linolenic fatty acid	C 18 : 3	0.51 ± 0.010
Arakhichidic fatty acid	C 20 : 0	5.42 ± 0.007	Arakhichidic fatty acid	C 20 : 0	3.30 ± 0.008
Behenic fatty acid	C 22 : 0	-	Behenat fatty acid	C 22 : 0	-
EPA	C 20 : 5	6.69 ± 0.002	EPA		4.89 ± 0.072

Table 4. Fatty acid profile for locally-produced *Artemia* sp. with *Chaetoceroscalcitrans* and *Skeletonemacostatum* feeding

Fatty acid		Percentage of fatty acid composition (± sd) without treatment	Percentage of fatty acid composition (± sd) with *Chaetocero scalcitrans* feeding	Percentage of fatty acid composition (± sd) with *Skeletonema costatum* feeding	Percentage of fatty acid composition (± sd) with *Chaetoceros calcitrans* and *Skeletonema costatum* feeding
Kaprilic fatty acid	C 8 : 0	-	0.30 ± 0.011	-	-
Kapric fatty acid	C 10 : 0	-	0.05 ± 0.009	-	0.30 ± 0.04
Lauric fatty acid	C 12 : 0	-	0.11 ± 0.015	0.08 ± 0.027	-
Miristic fatty acid	C 14 : 0	3.74 ± 0.011	10.62 ± 0.018	2.04 ± 0.046	0.66 ± 0.011
Palmitic fatty acid	C 16 : 0	9.14 ± 0.017	12.86 ± 0.016	6.69 ± 0.009	11. 58 ± 0.110
Palmitoleic fatty acid	C 16 : 1	9.84 ± 0.027	10.98 ± 0.019	7.15 ± 0.009	3.78 ± 0.027
Stearat fatty acid	C 18 : 0	3.80 ± 0.019	4.22 ± 0.032	3.89 ± 0.016	4.47 ± 0.029
Oleic fatty acid	C 18 : 1	18.98 ± 0.014	25.09 ± 0.012	18.05 ± 0.014	22.56 ± 0.008
Linoleic fatty acid	C 18 : 2	2.87 ± 0.009	4.65 ± 0.017	1.08 ± 0.009	7.24 ± 0.009
Linolenic fatty acid	C 18 : 3	0.51 ± 0.010	0.17 ± 0.028	2.47 ± 0.019	-
Arakhichidic fatty acid	C 20 : 0	3.30 ± 0.008	2.92 ± 0.043	6.34 ± 0.024	27.28 ± 0.005
Behenic fatty acid	C 22 : 0	-	2.09 ± 0.015	-	-
EPA		4.89 ± 0.072	4.67 ± 0.006	4.93 ± 0.031	3.31 ± 0.032

Table 5. Essential amino acid profile for locally-produced and imported *Artemia* sp.

Essential amino acid	Result (ppm)		Ratio (ppm)
	Imported *Artemia* sp. (± sd)	Locally-produced *Artemia* sp. (± sd)	
L- Histidine	392.15 ± 0.030	264.04 ± 0.027	128.11 (I)
L-Threonin	7604.39 ± 0.016	7962.22 ± 0.030	0.358 (L)
L- Arginine	2770.24 ± 0.088	2850.11 ± 0.015	0.008 (L)
L- Methionine – L Thryptophan	4608.93 ± 0.015	3816.42 ± 0.044	0.79 (I)
L- Valin	525.3 ± 0.021	505.18 ± 0.030	20.12 (I)
L- Phenylalanin	624.45 ± 0.023	701.62 ± 0.014	77.17 (L)
L- Isoleucine	1256.00 ± 0.048	1324.49 ± 0.086	0.06 (L)
L- Leucine	960.69 ± 0.034	1061.15 ± 0.042	100.46 (L)
L-Lycine	130.96 ± 0.043	131.50 ± 0.040	0.54 (L)

Skeletonema costatum does not affect the DHA content in locally-produced *Artemia* sp.

Essential Amino Acid Profile. The amino acid profile for locally-produced and imported *Artemia* sp. is given in Table 5. The fatty acid profile after feeding with both *Chaetoceros calcitrans* and *Skeletonema costatum* is given in Table 4. Our results indicated that the highest essential amino acid content in *Artemia* sp. was obtained when *Artemia* sp. was fed with a combination of both *Chaetoceroscalcitrans* and *Skeletonema costatum* (total of 18,912.62 ppm). The second highest was when *Artemia* sp. was fed with *Chaetoceros calcitrans* (12,756.78 ppm) and the least was when *Artemia* sp. was fed with *Skeletonema*

Table 6. Essential amino acid profile for locally-produced *Artemia* sp. with *Chaetoceros calcitrans* and *Skeletonema costatum* feeding

Essential amino acid	Result			
	Locally-produced *Artemia* sp. without treatment (ppm)	Locally-produced *Artemia* sp.with *Chaetoceros calcitrans* feeding (ppm)	Locally-produced *Artemia* sp.with *Skeletonema costatum* feeding (ppm)	Locally-produced *Artemia* sp. with *Chaetoceros calcitrans* and *Skeletonema costatum* feeding (ppm)
L- Histidine	264.04 ± 0.027	279.10 ± 0.044	353.86 ± 0.018	293.59 ± 0.032
L-Threonine	7962.22 ± 0.030	5.237.96 ± 0.046	7233.97 ± 0.031	7990.32 ± 0.025
L- Arginine	2850.11 ± 0.015	1209.03 ± 0.014	2440.35 ± 0.016	2700.17 ± 0.019
L- Methionine – L-Thryptophan	3816.42 ± 0.044	2658.07 ± 0.0088	3402.62 ± 0.032	4366.48 ± 0.018
L- Valin	505.18 ± 0.030	630.51 ± 0.022	464.19 ± 0.022	521.85 ± 0.014
L- Phenylalanine	701.62 ± 0.014	459.30 ± 0.041	596.99 ± 0.014	645.43 ± 0.021
L- Isoleusine	1256.00 ± 0.048	974.49 ± 0.047	939.02 ± 0.013	1211.36 ± 0.030
L- Leucyne	1061.15 ± 0.042	213.82 ± 0.028	807.01 ± 0.023	1053.15 ± 0.045
L-Lycine	130.96 ± 0.043	1094.50 ± 0.032	72.51 ± 0.015	130.27 ± 0.034

costatum (16,310.52 ppm). The essential amino acid profile after feeding with both *Chaetoceros calcitrans* and *Skeletonema costatum* is given in Table 6.

Our results also showed that the highest amino acid (threonina) was evidence in *Artemia* sp. when it was fed with a combination of *Chaetoceros calcitrans* and *Skeletonema costataum*, while the lowest amino acid (lysine) was observable in *Artemia* sp. when it was fed with *Skeletonema costatum*.

Water Quality. The water used for the research was within the proper quality. The temperature was between 25.0-30.5 °C, pH was around 7.8-8.9, the salinity was in the range of 30-31 ppt, and the DO was at 3.0-4.4 mg/L.

DISCUSSION

***Artemia* sp. Quality.** The quality of *Artemia* sp. depends largely on the hatching rate of the cysts to the nauplii. This is in line with the statement of Riberio and Jones (2008) stated that hatching rate is the defining factor of *Artemia* sp. quality. The faster *Artemia* sp. hatches, the better the quality is. Hatching Rate (HR) is influenced by some factors including *Artemia* sp. quality, hatching time, the number of cysts, and condition of the medium (whether aeration occurs or not). Good, fast-hatching *Artemia* sp. is the most suitable to feed fish and shrimp larvae.

Proximate analyses showed that nutritional content of locally-produced *Artemia* sp. was higher than imported *Artemia* sp., which is currently used in shrimp hatcheries. This result is further emphasized by the findings of Sudaryono (2006) and Suhartono *et al.* (2008), which indicate that nutritional content of locally-produced *Artemia* sp. is higher than that of its imported counterpart now being used in many fish and shrimp hatcheries.

Fatty Acid Profile. The highest saturated fatty acid (palmitic acid) in *Artemia* sp. was obtained after feeding with *Chaetoceros calcitrans* (12.86%). Based on a research by Brown (2002), palmitic acid is the most commonly found saturated fatty acid (around 15-50%). Then second on the order is stearate, at 25%. Palmitic acid is a saturated fatty acid that serves as energy storage used for SAFA or fatty acid biosynthesis. Meyer (2004), Benjamin and Olivia (2007) mentioned in their experiments that palmitic fatty acid is the substrate of SAFA fatty acid biosynthesis.

The highest unsaturated fatty acid in *Artemia* sp. after feeding with *Chaetoceros calcitrans* was oleat (25.09%). Oleate is the substrate that forms the long PUFA fatty acid chain. Pratiwi *et al*. (2009) stated that oleate is the substrate in the process of denaturation and catalyst elongation. PUFA biosynthesis starts from oleate, and then into linoleum as a substrate for the formation of the omega 6 long chains, and finally into linolenum that functions as the substrate for the formation of the omega 3 long chains. The process of PUFA formation involves a series of denaturation processes and elongation of catalysts with the help of desaturation and elongation enzymes (Fabergas *et al.* 2009).

The unsaturated fatty acid DHA is the only drawback in *Artemia* sp.; this lack cannot be compensated with the feeding of *Chaetoceros calcitrans* and *Skeletonema costatum*. This fact was evidence in the fatty acid profile analysis that did not detect any DHA. This might due to the fact that the feeds, *Chaetoceros calcitrans* and *Skeletonema*

costatum, were harvested in their final/declining phase, when they did not have enough DHA content for *Chaetoceros calcitrans* and only 0.9% for *Skeletonema costatum*. DHA in the feed is only available during the stationary or near life-end phase (Pratiwi *et al.* 2009).

The most important fatty acids for the growth of fish and shrimp larvae are EPA (3.2%) and DHA (0.9%) (Brown 2002). Our result on the availability of EPA in locally-produced *Artemia* sp. fed with *Chaetoceros calcitrans* and *Skeletonema costatum* showed that *Artemia* sp. meets the need of EPA for *Vanamme* shrimp larvae.

Essential Amino Acid Profile. *Artemia* sp. is high in protein content that in turn makes it rich in essential amino acid. Hence, it cannot be replaced with any other feed (Khoi 2009; Vilchlis 2010). Analyses of essential amino acid profile revealed that locally-produced *Artemia* sp. is rich in threonine (7990.32 ppm) when fed with a combination of *Chaetoceros calcitrans* and *Skeletonema costatum*. Threonine amino acid serves to build the frame for vitamin formation, because it has nucleate acid that functions to bond ions required for enzyme reactions. Threonine also helps to prevent fat sedimentation (Ming 2009; Herawati *et al*. 2012). Brown (2002) mentioned that threonine is essential for ion bonding in enzyme reaction for protein.

Our analyses also revealed that the lowest essential amino acid was lysine (72.51 ppm) in *Artemia* sp. fed with *Skeletonema costatum.* Lysine functions to build the frame for vitamin B1, serves as an antivirus, helps calcium absorption, forms antibody and hormones, stimulates appetite, and also facilitates the carnitine process to convert fatty acid into energy (Herawati *et al.* 2012). Brown (2002), Harrison *et al.* (2008), and Herawati (2013) stated that the most important function of lysine is to stimulate appetite, and to promote the production of carnitine to convert fatty acid into energy.

The other essential amino acids that are important for the growth of fish and shrimp larvae are valine, leucine, iso-leucine and lysine (Brown 2002; Herawati 2013). Valine, leucine and leucine help with the muscle contraction mechanism to transport, store, and manage nutrition in the body.

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